TOLERANCE TO THE OCULAR AND CARDIOVASCULAR EFFECTS OF REPEATED LOW-LEVEL EXPOSURE TO SARIN VAPOR

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ABSTRACT

Inhibition of acetylcholinesterase (AChE) by the nerve agent sarin (GB) results in accumulation of acetylcholine and excessive cholinergic stimulation. Little data exists in the literature regarding the effects of low-level repeated exposure to GB vapor. In the present study, male rats were exposed to multiple low-level inhalation exposures to GB vapor. The data from the present study demonstrate that multiple inhalation exposures to GB vapor result in tolerance to the ocular and autonomic effects of GB vapor. While the observed decrease in the potency of GB in rats cannot be attributed to a reduction in the inhibitory effect of GB, additional experiments are needed to determine the exact mechanism of the tolerance observed.

INTRODUCTION

While the acute and chronic effects of exposure to lethal doses of sarin (GB) are well documented, the effects of low-level repeated exposures remain uncertain. Some studies have found effects of low-level exposure of GB that persist after the cessation of exposure. In many of these studies GB liquid was injected subcutaneously or intramuscularly. However, the route of exposure is an important determinant of the effect observed. Arguably, the most relevant route of exposure for GB is vapor inhalation. Therefore, the present study was undertaken to investigate the ocular and cardiovascular effects of repeated low-level inhalation exposures to GB vapor.

METHODS

Exposure protocol.

Male Sprague-Dawley rats (200-250 g) were exposed to GB vapor (3.93 ± 0.05 mg/m^3) for 1 hour on each of 3 consecutive days. GB vapor was generated using a spray atomization system in a 750-L dynamic airflow chamber.

Cardiovascular measurements.

Telemetric transmitters were surgicallly implanted into rats a week prior to exposure to GB. Blood pressure (BP), heart rate (HR), and a lead II electrocardiogram (ECG) were recorded telemetrically (sampling rate = 5000 Hz; filter cutoff = 1250 Hz) pre-, during, and post-exposure (n=3). Time and frequency domain indices of HR variability were calculated using both Fourier and wavelet techniques. The ratio of low-frequency power (LF; 0.27-0.74 Hz) to high-frequency power (HF; 0.74-3.85 Hz) was used as an indicator of the balance of tone between the parasympathetic and sympathetic nervous systems. Left ventricular posterior wall thickness and cardiac fractional shortening were assessed using two-dimensional guided M-mode echocardiography before exposure to GB, and at 1 week and 1 month post-exposure. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were determined in whole blood in air- and GB-exposed animals following each exposure using a modified Ellman assay.

Ocular measurements.

Pupil and iris measurements were determined by infrared pupillography using an infrared capable video camera equipped with a 75-mm/F2.7 lens, an image acquisition PC card, and custom analysis software. This technique has been described previously (Miller et al. 2003). All images were taken at <10 foot-candles. The ratio of pupil radius to iris radius was used as a measure of the miotic effect of GB vapor. AChE and BChE activities in the vitreous humor, the anterior eye, and the posterior eye were determined in air- and GB-exposed animals following each exposure using a modified Ellman assay.

RESULTS

BP, HR, and the standard deviation of HR were not significantly altered during or following the exposures. However, the incidence of transient ventricular asystole and ventricular premature beats (VPBs) was increased following each of the three exposures.

Additionally, the ratio of low frequency power to high frequency power (LF/HF) decreased following the first exposure (Figure 1). However, subsequent exposures did not produce the same magnitude of response elicited by the first exposure.

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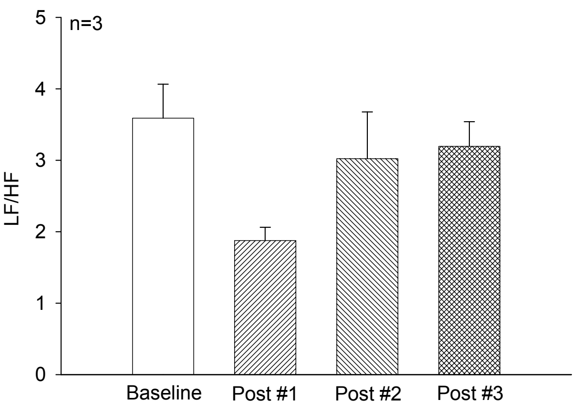

Figure 2 – Effect of multiple GB exposures on heart rate variability. The ratio of low-frequency power (LF) to high-frequency power (HF), calculated using a db7 wavelet transform, decreased following the first exposure. However, subsequent exposures did not produce the same magnitude of effect.

There was no change in left ventricular posterior wall thickness and cardiac fractional shortening at 1 week or 1 month post-exposure when compared to baseline measurements. Whole blood AChE activity in GB-exposed rats was 30% of the level in air-exposed rats following exposure 1. A similar level of inhibition was present following exposures 2 and 3. Whole blood BChE activity was not different between air- and GB-exposed animals following any of the exposures.

Following the first and second exposures, all GB-exposed rats had pinpoint pupil (>97% reduction in the ratio of pupil radius to iris radius) (Figures 2B and 2C). However, following the third exposure, there was only a 45-50% reduction in the ratio of pupil radius to iris radius (Figure 2D). The rate of recovery from GB-induced miosis was also increased following the second exposure.

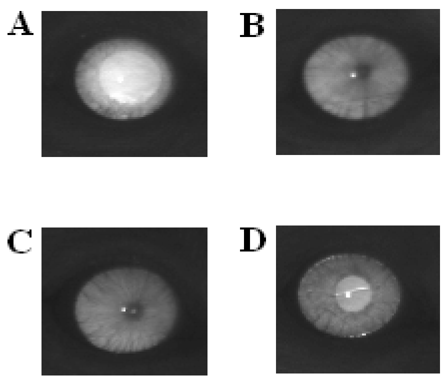

Figure 2 – Representative pupil images taken (A) pre-exposure #1, (B) 15 minutes post-exposure #1, (C) 15 minutes post-exposure #2, and (D) 15 minutes post-exposure #3.

AChE activity in the vitreous humor, anterior eye, and posterior eye was inhibited to a similar degree following each of the 3 exposures. BChE in the vitreous humor, anterior eye, and posterior eye was not different from air-exposed animals following the first 2 exposures. Following the 3rd exposure, there was a significant decrease in BChE in all three areas of the eye.

DISCUSSION

The data from the present study demonstrate that multiple inhalation exposures to GB vapor result in a decrease in the potency of GB in rats. This tolerance developed at a dose of GB that produced no overt clinical signs of intoxication other than miosis.

Tolerance to the effect of GB on the autonomic nervous system developed following the first exposure, whereas tolerance to the miotic effect of GB vapor developed following the 2nd exposure. AChE and BChE activity in whole blood, as well as in ocular tissue, did not increase throughout the exposure sequence, suggesting that the miotic and autonomic tolerance observed cannot be attributed to a reduction in the inhibitory effect of GB. The tolerance to the autonomic effects of GB vapor has not been previously reported, and is currently being investigated further. Regarding the miotic tolerance observed, a similar result has been reported previously for the nerve agent soman (Soli et al. 1980). Miosis has been proposed as an indicator of exposure to nerve agents. However, the results of the present study suggest that individuals who have been previously exposed to an OP compound may demonstrate tolerance to the effects of GB exposure, including miosis, upon subsequent exposure. Thus, in these individuals, miosis may not be a consistent a marker of exposure.

While the mechanism mediating the miotic and autonomic tolerance observed following multiple inhalation exposures to the nerve agent GB remains uncertain, several possibilities can be excluded based on the results of the present study. However, additional studies are necessary to determine the timecourse of the tolerance and to determine if exposure to a particular OP compound results in miotic tolerance to other OP compounds.

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